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Poly (*N*-Isopropylacrylamide) Microgel-Based Assemblies for Organic Dye Removal from Water

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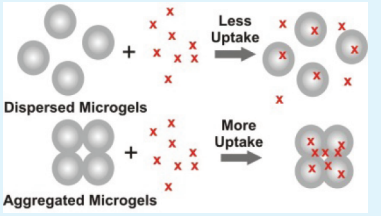
Supporting Information

ABSTRACT: Poly(*N*-isopropylacrylamide)-*co*-acrylic acid (pNIPAm-*co*-AAc) microgel assemblies (aggregates) were synthesized via polymerization of the cross-linker *N*,*N*′-methylenebisacrylamide (BIS) in the presence of microgels in solution. In this case, the microgels were entrapped in the polymerized cross-linker network. The aggregates were investigated for their ability to remove the organic, azo dye molecule 4-(2-hydroxy-1-napthylazo) benzenesulfonic acid sodium salt (Orange II) from water at both room and elevated temperatures. These results were compared with unaggregated microgels that were previously reported (Parasuraman, D.; Serpe, M. J. *ACS. Appl. Mater. Interfaces* **2011**, 3, 2732.). It was found that the removal efficiency increased at elevated temperature, most likely due to the thermoresponsive nature of the pNIPAm-based aggregates, which expel water of solvation and deswell at higher temperature and reswell when they are cooled back to room temperature. Furthermore, increasing the number of cycles the aggregates are heated and cooled enhanced the percent removal of the dye from water. We also evaluated the effect of increasing cross-linker concentration on the removal efficiency, where we found the removal efficiency to increase with increasing cross-linker concentration in the aggregates. The maximum removal efficiency reached by the microgel aggregates at elevated temperatures was calculated to be 73.1%. This enhanced uptake is due to the presence of larger internal volume between the microgels in the aggregates, which the individual microgels lack. Control studies reveal that the structure and hydrophobicity of the aggregates lead to the enhanced uptake efficiencies and is not due to the presence of BIS alone. We determined that aggregates leak 75.6% of the dye that was originally removed from solution. The removal of Orange II by the aggregates at room temperature was fit by a Langmuir sorption isotherm.



KEYWORDS: water remediation and contamination, poly(*N*-isopropylacrylamide)-*co*-acrylic acid microgel aggregates, thermo- and stimuli-responsive polymeric materials, azo dyes, pNIPAm

INTRODUCTION

Synthetic dyes are used daily, in large quantities by several industries like leather tanning, textile, paper production, and food technology.[2−7] Out of these, the textile industry alone uses several different dyes, 30% of which are classified as reactive dyes. These dyes have reactive functional groups, such as azo, anthraquinone, and oxazine, that get activated and react with the fibers of the material to be dyed.[8] Although the exact amount of dyes produced is not available, it is estimated that about 10 000 tons of dyes are being produced every year.[2] Of all the reactive dyes, ca. 60% are azo dyes.[2,8] Because such large quantities of azo dyes are being produced and used daily, and they are known to transform to carcinogenic aromatic amines in the environment,[9] their incorrect disposal is a major environmental concern and can affect human and animal health.[2]

There are several reports of physicochemical and biological treatments for removal of dyes from industrial effluents, but often these methods are expensive and not easy to use/implement.[10−18] One of the most commonly used techniques for treating drinking water is coagulation and flocculation. This involves the addition of specific compounds (coagulants) to the contaminated water, followed by sedimentation.[21] This approach has been shown to remove a variety of dyes from water including azo and other reactive dyes.[19,20,22−24] Although this technique is widely used for water remediation, it can also lead to secondary pollution from the excessive use of coagulant compounds.[25] A variation of coagulation induced by the addition of coagulant compounds to contaminated water is to generate the coagulant at an anode. This technique, called electrocoagulation, has been shown to remove Samaron Yellow and Eriochrome Black T from water.[17,25] Dyes like C.I. Reactive Black 5 and C.I. Vat Yellow 4 in industrial wastewater have been removed via flocculation.[26−28] Finally, adsorption of organic contaminants onto activated carbon is exploited frequently for removing contaminants from water.[29,30]

In the current study, we use particles composed of responsive polymers for water remediation. Responsive polymers "react" to an external stimulus by changing their physical and/or chemical state. These polymers can be made sensitive to a variety of stimuli including: temperature, pH, ionic strength, light, force and analyte concentration.[31−38] Poly(*N*-isopropylacrylamide) (pNIPAm) is arguably the most well studied responsive polymer.[39−47] PNIPAm is fully water-soluble, and exists as a random coil at $T < \sim 32$ °C.

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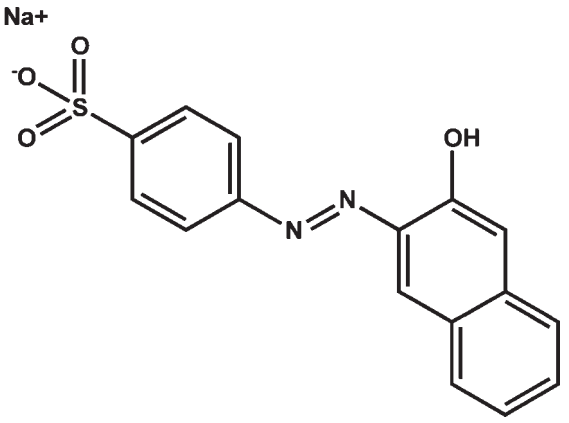

Figure 1. Structure of Orange II.

As the temperature of the water is increased to $T > \sim 32$ °C, pNIPAm undergoes a random coil to globule transition, expelling much of its solvating water as a result. The temperature that this occurs at is called the lower critical solution temperature (LCST).[40,41,44,45]

Several reports detail the synthesis of colloidal NIPAm particles.[40,41,43,45−51] These particles, often referred to as microgels, are highly porous, water-soluble and are thermoresponsive. That is, pNIPAm microgels expel their water of solvation and decrease in diameter at $T \gtrsim 32$ °C, and reswell at $T \lesssim 32$ °C. By simply adding different comonomers to the microgels during synthesis, they can be made to respond to other stimuli.[43,46−49,52,53] The most common comonomer for this purpose is acrylic acid (AAc), which has a $pK_a \approx 4.25$. Therefore, at pH > pK_a, the microgels swell due to Coulombic repulsion in the microgel network. This fact also hinders the thermoresponsivity of pNIPAm-*co*-AAc microgels.[43,54−57]

In previous reports, pNIPAm-based hydrogels have been employed to determine the partition coefficient of 4-(2-hyrodxy-1-napthylazo) benzenesulfonic acid sodium salt (Orange II) and methylene blue in the system at different temperatures. Specifically, Orange II was reported to have more permeability through the hydrogels, when temperature was raised above 32°C.[56,58,59] In other reports pNIPAm-based hydrogels and microgels have been used for removal of heavy metal ions like Pb (II) and Cu (II) and dyes like Nile red, brilliant green, and brilliant cresyl blue for water remediation[60−63] Recently, we reported on the use of pNIPAm based microgels for the removal of Orange II, Figure 1, from water. Orange II has been considered as a contaminant from various industrial effluents and treated using physical and chemical methods.[64−66] Using this system, we were able to remove a maximum of 56.6% of the dye from water, and the microgels were able to retain 74.4% of the Orange II.[1]

This submission expands on our previous study of Orange II uptake by microgels, by investigating the effect of microgel aggregation on the removal efficiency. We synthesized aggregates of pNIPAm-based microgels, by polymerizing *N*,*N*′-methylenebisacrylamide (BIS) in the presence of an aqueous solution of microgels and use them to remove Orange II from aqueous solution. The BIS polymer network is able to entrap the microgels, forming clusters of "aggregated" microgels. We assessed the efficiency of removing Orange II from solution as a function of BIS concentration in the aggregates, and observed that the removal efficiency increased as the concentration of BIS in the aggregates increased. Similarly, we investigated how the removal efficiency depended on the concentration of aggregates in solution, and found it to increase with aggregate concentration, but once a certain concentration was reached, the uptake leveled off (Langmuir-like sorption). We also found the removal efficiency to depend on the solution temperature, heating the solution yielded more uptake. Overall, a maximum removal efficiency of 73.1% was achieved for the aggregates, in contrast to 56.6% by the unaggregated microgels.[1] We attribute this enhanced uptake to the higher interstitial volume present between the microgels of the aggregates (structure), in addition to and the necessarily enhanced hydrophobicity of the aggregates. We also determined the overall retention efficiency of these microgel aggregates by monitoring the amount of Orange II that leaked from them after uptake. The microgel aggregates retained 75.6% of the dye that was initially removed from the solution.

The results from our studies reveal that the microgel aggregates are capable of removing significant amounts of Orange II from water in a fast, straightforward, and inexpensive manner. In the future, we will develop extraction and isolation techniques to investigate the reusability of these aggregates for further remediation. Furthermore, we will explore chemical modification of the aggregates to specifically remove certain classes of contaminants, e.g., metals, pharmaceuticals, charged contaminants, and naphthenic acids. These results, while interesting for water remediation, will also aid us in our pursuit of controlled/triggered drug delivery systems.[67]

MATERIALS AND METHODS

Materials. The monomer *N*-isopropylacrylamide was purchased from TCI (Portland, OR) and purified by recrystallization from hexanes (ACS reagent grade, EMD, Gibbstown, NJ). *N*,*N*′-Methylenebisacrylamide (BIS) (~99%), acrylic acid (AAc) (~99%), and ammonium persulfate (APS) (~98%) were obtained from Sigma-Aldrich (Oakville, Ontario) and were used as received. Orange II was obtained from Eastman Organic Chemicals (Rochester, New York). All the phosphate salts used for preparing buffer solutions of pH 7, with an ionic strength of 0.235 M, were obtained from EMD and were used as received. Deionized (DI) water with a resistivity of 18.2 MΩ cm was obtained from a Milli-Q Plus system from Millipore (Billerica, MA), and filtered through a 0.2 μm filter prior to use. Microgel samples were lyophilized using a VirTis benchtop K-manifold freeze-dryer (Stone Ridge, New York).

Synthesis of Microgels. PNIPAm-*co*-AAc microgels of ~1 μm diameter were prepared by a surfactant-free, free radical precipitation polymerization as described previously.[43] The total monomer concentration was 140 mM, and was 85% *N*-isopropylacrylamide (NIPAm), 5% *N*,*N*′-methylenebisacrylamide (BIS) cross-linker and 10% acrylic acid (AAc) . The microgels will be indicated as pNIPAm-*co*-10AAc, to show that they contain 10% AAc (the microgel composition is assumed to match the monomer "feed" composition). The monomer, NIPAm (11.9 mmol) and the cross-linker, BIS (0.700 mmol), were dissolved in deionized water (75 mL) in a beaker with stirring. The mixture was filtered through a 0.2 μm filter affixed to a 20 mL syringe into a 250 mL, 3-neck round-bottom flask. An additional 24 mL of deionized water was used to wash the beaker, which was filtered and transferred to the mixture in the round-bottom flask. The flask was then fitted with a condenser, temperature probe (thermometer), stir bar, and a N_2 inlet. The temperature was set to 65°C and N_2 was bubbled through the solution for ~1 h, after which AAc (1.40 mmol) was added to the mixture and allowed to stir for a few minutes. To this, 0.197 mmol APS in 1 mL of DI water was added. The mixture was allowed to stir

for 4 h, under a N_2 atmosphere. The solution was allowed to cool, while stirring overnight.

Following stirring overnight, the microgels were filtered through a type 1 Whatman filter paper, which was then rinsed with deionized water. The microgels were cleaned via centrifugation to remove unreacted monomer and cross-linker, as well as linear polymer from the microgels. To do this, the microgel solution was separated into 15 mL centrifuge tubes obtained from Corning Incorporated (Corning, NY) (~ 12 mL microgel solution/tube) and centrifuged at a speed of ~8400 relative centrifugal force (rcf) in a Baxter, Biofuge 17R (Mount Holly, NJ) at 23 °C, for 30 min. Centrifugation yielded a pellet of microgels at the bottom of the centrifuge tube, and the supernatant was removed. Approximately 12 mL of fresh DI water was added and the microgel pellet was redispersed using a Fisher Vortex, Genie 2 vortexer (Pittsburgh, PA). This cleaning protocol was repeated six times.

Synthesis of Microgel Aggregates. Microgel aggregates were synthesized using three different concentrations of BIS, 2, 10, and 15 mg/mL of total reaction solution (100, 500, and 750 mg total BIS mass, respectively). The first set of aggregates were prepared by adding 10 mL of the above synthesized and cleaned microgels to a filtered solution (filtered through 0.2 μm filter affixed to a 20 mL syringe) of 100 mg of BIS in 39 mL of deionized water, to a beaker and stirred. This solution was transferred into a 250 mL 3-necked round-bottom flask that was fitted with a condenser, thermometer, stir bar, and a N_2 inlet. The temperature was set to 65 °C and N_2 was bubbled through the solution for ~1 h. After 1 h, a 1 mL aqueous solution containing 0.0175 mmol of APS was added to this mixture and left to stir for 4 h, under a N_2 atmosphere. The solution was allowed to cool with stirring overnight. This process was repeated for the 10 and15 mg/mL BIS aggregate samples. The microgel aggregates were cleaned using the same protocol as mentioned in the microgel synthesis section, but without filtering.

Orange II Uptake. The individual aggregate solutions were lyophilized to yield a powder, and stock solutions of each were made to contain 5.2 mg aggregates/mL by weighing out 52.1 mg of each aggregate sample into a volumetric flask, and diluting to 10 mL with a pH 7 buffer solution of 0.235 M ionic strength. A stock solution of 0.023 M Orange II in deionized water was prepared. Using a micropipet, 300 μL of the respective aggregate solutions and 15 μL of Orange II solution was added to a 15 mL centrifuge tube (Corning Inc., Corning, NY) and diluted to 3 mL with the pH 7 buffer solution (final concentrations of aggregates and Orange II were 521 μg/mL and 114 μM, respectively). This sequence of addition of buffer after exposing the dye to the aggregates was maintained for all experiments. This solution was allowed to sit for five minutes and then centrifuged for 30 min, at ~8400 rcf to pack the aggregates to the bottom of the centrifuge tube. This centrifugation time was used to ensure that all the dispersed microgels were removed from solution (as confirmed from differential interference contrast microscopy, data not shown). The supernatant was carefully removed from the tube without disturbing the pellet of aggregates packed at the bottom of the centrifuge tube and transferred to a quartz cuvette. The absorbance was measured using a HP8452A UV−vis spectrophotometer with a diode array detector (Agilent Technologies, Inc., Santa Clara, CA). The initial concentration of Orange II for all the uptake studies was maintained at 114 μM and it was observed that the pH of Orange II solution was unaffected by the addition of aggregates. The initial absorbance of Orange II was measured in the absence of the aggregates. This measurement was performed before all the studies. It was also observed that the centrifuge tube did not have any effect on the initial absorbance of Orange II, i.e., the tube does not interact with Orange II. The absorbance maximum for Orange II was observed at 486 nm.

To study the uptake of Orange II as a function of temperature, we held the solution of Orange II and aggregates at 50 °C for different intervals of time (microgels deswell) and then cooled them to room temperature (microgels reswell). This solution was then centrifuged and the supernatant was used to perform UV−vis studies.

Orange II Leaking Studies. To evaluate the ability of the microgels to retain Orange II, we scaled up the concentration and volumes from the previous section three times. So in this case, 900 μL of microgels were exposed to 114 μM Orange II in a total volume of 9 mL using the same buffer that was used for the uptake studies. This scaling up was done to get a detectable absorbance signal from the solutions after leaking. As for the Orange II removal studies above, the solution was allowed to sit for five minutes followed by centrifugation for 30 min. The supernatant solution was then carefully removed without disturbing the microgels packed at the bottom. To this tube, 9.0 mL of fresh buffer solution (the same buffer that was used for the uptake studies) was added and then the microgels were redispersed by vortexing. This solution was immediately divided into nine, one mL samples in 1.5 mL Eppendorf tubes obtained from Fisher Scientific, (Ottawa, ON) and the solutions were allowed to incubate for various intervals of times. For example, immediately following splitting up the original solution, one tube was centrifuged for 30 min and the supernatant solution removed and UV−vis performed on the supernatant solution, this was considered $t = 0$. We allowed the other tubes to incubate for 10, 20, 30, 40, 50, 60, 70, and 80 min, respectively, before each was centrifuged.

Counting of Aggregates. Solutions containing 41.6 μg/mL of the 100, 500, and 750 mg BIS aggregates (2, 10, and 15 mg/mL) were prepared. A drop of these samples were placed on a microscope coverslip (25 mm × 25 mm, Fischer Scientific, Ottawa) and pictures of 20 random areas of the sample were captured using a Olympus IX71 inverted microscope (Markham, Ontario) fitted with a 100× oil immersion objective, differential interference contrast (DIC) optics and an Andor Technology iXon+ camera (Belfast, Ireland). There were different sizes of aggregates in these samples that were designated as "big", "medium", and "small". When making the designation of size, we measured the apparent diameter of the aggregates in two approximately orthogonal directions. If the two dimensions were ≥0.6 μm × 1.5 μm but <5.7 μm × 4.5 μm the aggregate was considered small, medium aggregates had dimensions that were ≥5.7 μm × 4.5 μm but <10.3 μm × 11.5 μm, and large aggregates had dimensions that were ≥10.3 μm × 11.5 μm. Any aggregate <0.6 μm × 1.5 μm was not counted, therefore single particles were not accounted for. A micrometer scale of 50 divisions, each 2 μm in length (Edmund Optics, NJ), was used to measure the sizes of these aggregates.

RESULTS AND DISCUSSION

Effect of Aggregation of Microgels. In our previous report, we evaluated the efficiency of the microgels to remove Orange II as a function of the concentration of the microgels in solution. We reported a maximum uptake of 29.5% for the pNIPAm-*co*-10AAc microgels at room temperature.[1] In this submission we monitored the removal efficiency of pNIPAm-*co*-10AAc microgel aggregates as a function of the amount of aggregates that were present in solution. To do this, the initial absorbance of a 3 mL solution of 114 μM Orange II in the absence of microgel aggregates was recorded. This was compared to the absorbance of the supernatant solution after addition of 100, 200, 300, and 400 μL (173, 347, 521, and 693 μg/mL) of the 500 mg BIS microgel aggregates to 114 μM Orange II, keeping the total volume of 3 mL consistent from run to run. A calibration curve (see the Supporting Information) was used to calculate the number of moles of Orange II in solution before and after addition of aggregates. The conditions used here are the same as those for the unaggregated microgels in our previous report.[1] Figure 2 shows the percent uptake as a function of concentration

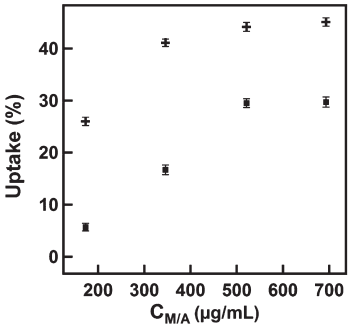

Figure 2. Uptake of Orange II as a function of concentration of microgels (C_M), shown by (■), and concentration of microgel aggregates (C_A), shown by (+). The total volume of all the solutions was maintained at 3 mL in pH 7 buffer. Each point on the plot represents an average of three replicate experiments of uptake studies and the error bars denote the standard deviation.

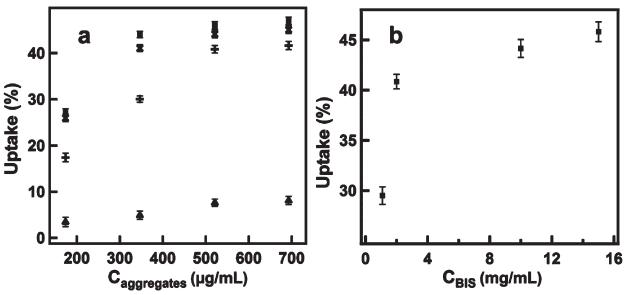

Figure 3. Uptake of Orange II as a function of (a) concentration of aggregates from the synthesis using (+) 100 mg, (×) 500 mg, and (■) 750 mg BIS and (▲) control experiment using 500 mg BIS aggregates plus unaggregated microgels; (b) concentration of BIS in the aggregates. (b) First point in the plot the uptake from unaggregated microgels. Each point on the plot represents an average of three replicate experiments of uptake studies and the error bars denote the standard deviation.

of unaggregated pNIPAm-*co*-10AAc microgels (from our previous report) and the percent removal as a function of concentration of the microgel aggregates. The figure shows that the maximum removal efficiency from the aggregates was 45.1% as opposed to 29.5% achieved from the unaggregated microgels. This shows that the aggregation of microgels, significantly improved the removal efficiency. Furthermore, we calculated the mass of aggregated microgels that would need to be weighed out to result in the same number of microgels in the unaggregated microgel experiment.[1] That is, for a given mass, there are more microgels in the unaggregated sample, than in the aggregated sample. This is because the mass in the aggregated sample comes from a combination of BIS and microgels, while all the mass in the unaggregated case comes from the microgels alone (the active component). It was found that we had to prepare much higher concentrations of the aggregates in order to equal the particle number in the unaggregated sample. The data for the uptake studies obtained from these concentrations shows that a maximum of 69% removal efficiency is attained at room temperature after an exposure time of five minutes (see the Supporting Information). So, a sample of aggregated microgels containing the same number of microgels as in the unaggregated state results in ~43.1% increase in uptake. This is a significant improvement compared to the unaggregated case, and supports the hypothesis that the aggregated structure is leading to enhanced removal efficiency. This enhancement can be due to: (1) the aggregated microgels have more volume than unaggregated microgels because of the interstitial space between the packed microgels, therefore providing a larger reservoir for Orange II to be trapped in; (2) the aggregates are more hydrophobic than the unaggregated microgels, enhancing the interaction of Orange II with the aggregates; and/or (3) the BIS itself is interacting with the Orange II and removing it from water. A control experiment was performed in which 500 mg BIS was weighed out, added to 40 mL DI water and the aggregation was performed using the protocol mentioned in the Experimental, but in the absence of microgels. After the aggregation of BIS, 10 mL of pNIPAM-*co*-10AAc was added to the mixture; this yields a solution that has the same amount of BIS and microgels as the standard aggregated sample. Uptake studies were performed by monitoring the removal efficiency as a function of the aggregate concentration. The data in Figure 3a show that the aggregates from the control experiment removed only 8.1% of the dye from the solution as opposed to 45.1% removal efficiency by the aggregated microgels. Hence, it is important to have microgels in their aggregated form to result in enhanced removal. This suggests that either the interstitial space argument and/or the hydrophobicity arguments can be valid.

Effect of BIS Concentration on Removal Efficiency. The concentration of BIS (cross-linker) was varied in the microgel aggregates and the uptake studies of Orange II were performed at room temperature for each of these systems. Figure 3b shows the percent uptake as a function of an increase in the BIS concentration in the microgel aggregates. The first point on the plot denotes the uptake percent (29.5%) after addition of 300 μL (521 μg/mL) of unaggregated microgels, as reported previously.[1] The remaining data points denote the uptake percent for 100 mg (2 mg/mL), 500 mg '(10 mg/mL), and 750 mg (15 mg/mL) BIS in the synthesis of aggregates for the same concentration (521 μg/mL) of aggregate addition to the Orange II. It was found that the uptake percent was 40.85% for the 100 mg BIS (2 mg/mL) microgel aggregates, and the uptake increased to 44.16% for the 500 mg BIS (10 mg/mL) microgel aggregates. This is only 1.67% lower than that for the 750 mg BIS (15 mg/mL) microgel aggregates.

The effect of increasing the concentration of the aggregates for the 100 mg (2 mg/mL), 500 mg (10 mg/mL) and 750 mg (15 mg/mL) BIS concentrations was also investigated. Figure 3 (a) shows that as the concentration of the aggregates increased from 173 μg/mL to 521 μg/mL, the percent uptake of Orange II increased from 17.4 to 40.9% for the 100 mg (2 mg/mL) BIS aggregates; 25.9 to 44.2% for the 500 mg (10 mg/mL) BIS aggregates, and 27.1 to 46.0% for the 750 mg (15 mg/mL) BIS aggregates. For all the aggregates, there was no significant increase in uptake as the concentration of aggregates was increased from 521 to 693 μg/mL. We attribute this behavior to an equilibrium process that exists in the system, i.e., only a certain number of moles can be removed from solution because of equilibrium (although drastic changes in concentration does result in enhanced uptake, as indicated above). The data from these experiments show that the maximum removal efficiency achievable for the microgel aggregates at room temperature improved the uptake efficiency by ~17%, compared to the unaggregated microgels.[1] For the remainder of the studies, we employed the 500 mg BIS (10 mg/mL) aggregates at a concentration of 521 μg/mL.

We hypothesize that the trend of increased uptake efficiency with increasing concentration of BIS in the aggregates could be due to: (1) a change in the hydrophobicity of the aggregates with BIS concentration used to form the aggregates; (2) an increase in the number of aggregates that are in solution; and/or 3) a difference in the size of the aggregates in the respective solutions, which would provide more interstitial volume for Orange II to be trapped. Because it was established above that it is important to have aggregates in solution to achieve enhanced uptake, it stands to reason that if one solution has more aggregates relative to the others, than it should exhibit enhanced uptake.

A counting experiment was conducted as outlined in the Experimental Section. In short, solutions containing 41.6 μg/mL of the respective 100, 500, and 750 mg BIS aggregates (2, 10, and 15 mg/mL) were prepared and the number of aggregates present in a given volume of these solutions was determined. We found that the total number of aggregates increased from 131 $\pm$ 12 to 265 $\pm$ 17 as the amount of BIS in the aggregates was increased from 100 mg to 750 mg (see the Supporting Information). This is supported by the fact that there were more free, unaggregated particles in the 100 mg BIS sample, compared to the 500 and 750 mg BIS aggregate samples; these were not accounted for in the counting process. Interestingly, when the amount of 100 mg BIS aggregates exposed to Orange II was doubled, to give the same number of aggregates as the 750 mg BIS sample, the uptake efficiency was very close to the uptake efficiency achieved for the 750 mg BIS sample (see Figure 3a). When aggregate size is considered, it was observed that there is a higher percent of big aggregates in the 750 mg BIS sample compared to the 100 mg BIS sample, which can also lead to enhanced uptake for the 750 mg BIS aggregates. But, if aggregate size was important, when the 100 mg BIS sample concentration was increased, the 750 mg BIS sample should still result in more uptake, which was not the case. Taken together, it appears that in the low aggregate concentration regime the enhanced uptake for the 750 mg BIS aggregates is due to the increased number of aggregates in the sample, because when the number of aggregates in 100 mg BIS sample was set equal to the 750 mg BIS sample, very similar uptake efficiencies were achieved. It should be noted though that while doubling the mass of the 100 mg BIS sample exposed to the Orange II to result in similar percent uptake as the 750 mg BIS samples, the 750 mg BIS aggregates are still much more efficient at removing Orange II from water on a per mass basis. In the high concentration, or the "equilibrium" regime, where aggregate number no longer matters, the 500 and 750 mg BIS aggregate samples still result in enhanced uptake efficiency, compared to the 100 mg BIS sample. This can be explained considering the hydrophobicity of these aggregates, which will affect the Orange II partitioning behavior, in turn influencing the equilibrium constant. So, higher BIS concentrations must result in a higher equilibrium constant, which is operative across all concentration, but most apparent in the high concentration (equilibrium) regime. Therefore, hydrophobicity (possibly influenced by aggregate size) and aggregate number play important roles in the uptake efficiency of the aggregates.

Effect of Temperature Cycling on Removal Efficiency. To monitor the effect of a single temperature cycle, we added 300 μL (521 μg/mL) of the 500 mg (10 mg/mL) BIS aggregates to 15 μL of Orange II in the same buffer solution as before (3 mL total volume), and heated the solution above 32 °C for different intervals of time and cooled it down to room temperature. Specifically, we exposed the microgel aggregates to the dye for five minutes and heated to 50 °C for 20, 40, 60, 90, and 120 min and the solution was cooled down to room temperature (~23 °C) for 30 min and centrifuged immediately. Figure 4 shows the trend for the percent uptake as a function of heating time. The percent uptake at time 0 corresponds to the uptake of the microgel aggregates at room temperature (44.2%). It was observed that the uptake of the dye increased significantly from 47.1% to 67.8% as the time of heating increased from 20 to 90 min. Also, there was minimal increase in the uptake when the solution was heated for 120 min (68.1%). The dye removal was also visually confirmed by comparing the intensity of the Orange II solution before and after treatment, Figure 5. As can be seen, the solution intensity for the heated sample is significantly reduced. A control experiment confirmed that high temperature alone did not significantly affect the absorbance of Orange II (see the Supporting Information). Hence the reduction in Orange II intensity was due to increased uptake due to the aggregate's thermoresponsive nature. Thermoresponsivity of our microgels at this pH was confirmed by performing light scattering experiments (see the Supporting Information).[1]

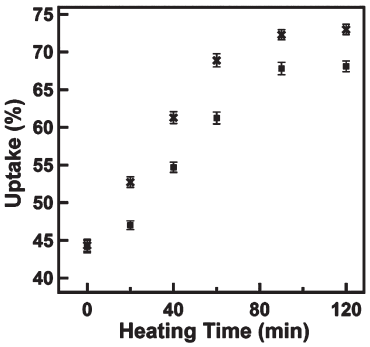

Figure 4. Uptake of Orange II as a function of the time the microgels were held at elevated temperature for (■) one and (×) two cycles. Each point on the plot represents an average of three replicate experiments of uptake studies and the error bars denote the standard deviation.

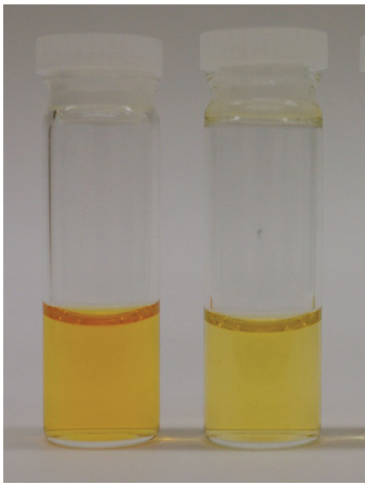

Figure 5. Solutions of 114 μM Orange II (left) before and (right) after the addition of 500 mg BIS aggregates (aggregate concentration of 521 μg/mL). Here, 2 heating cycles were performed for a total heating time of 90 min.

We also monitored the effect of the multiple heating and cooling cycles on the percent uptake. This was done by exposing

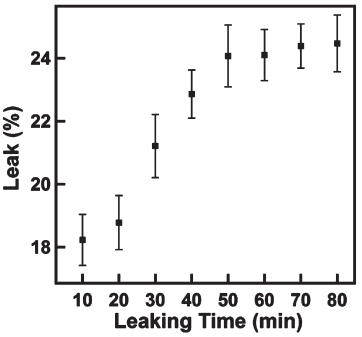

Figure 6. Percent leak of Orange II from the microgel aggregates as a function of leaking time. Each point on the plot represents an average of three replicate experiments of leaking studies and the error bars denote the standard deviation.

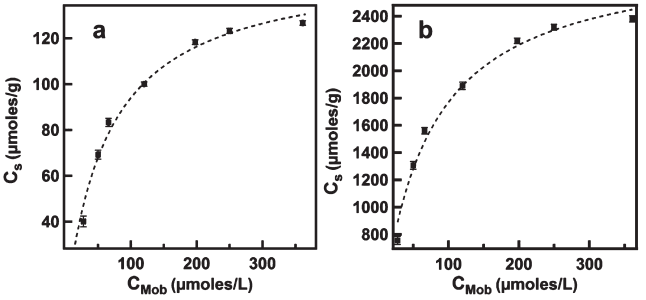

Figure 7. Langmuir sorption isotherms for (a) the removal of Orange II by the aggregates and (b) corrected to account for the microgels in the aggregates alone. C_{mob} is the concentration of the dye remaining in the aqueous phase and C_s is the concentration of Orange II sorbed on the aggregates/microgels. Each point on the plot represents an average of three replicate experiments and the error bars denote the standard deviation.

the 500 mg (10 mg/mL) BIS aggregates (521 μg/mL) to 15 μL Orange II in the same buffer solution and heated to 50 °C and cooled down to room temperature in two cycles, keeping the overall heating and cooling time the same as with the single heating cycle. For example, the solution of aggregates was exposed to the dye and heated to 50 °C for 10 min and cooled down to room temperature for 15 min and heated again to 50 °C for another 10 min and finally cooled for 15 min. So, overall the microgels were heated for 20 min and cooled for 30 min, but over two cycles. This protocol was repeated for all 40, 60, 90, and 120 min heating periods. Figure 4 shows how the extra heating cycle affects the removal of Orange II from water. It was observed that the maximum uptake of dye increased to 73.1% compared to the single cycle experiment where the maximum uptake was 68.1%. The overall percent uptake of the dye by aggregates was significantly higher than similarly treated unaggregated microgels.[1] We also investigated the effect of cycling between the high and room temperature three times and confirmed that there was no further improvement in the removal efficiency (see the Supporting Information).

Leaking of Orange II from Aggregates. In our previous work we reported that the pNIPAm-*co*-10AAc retained 74.4% of the dye that was removed from the aqueous solution.[1] We followed a similar protocol here to assess the percent of Orange II that was retained in the structures after uptake. This was done by exposing 900 μL of the microgel aggregates to 114 μM Orange II in a total volume of 9 mL pH 7 buffer. This solution was allowed to sit for five minutes and immediately centrifuged (as was done for all uptake experiments at room temperature). The supernatant solution was removed and the microgel aggregates packed at the bottom of the centrifuge tube were redispersed in 9 mL of fresh buffer using a vortexer. This solution was equally divided into 9 Eppendorf tubes and incubated for different intervals of time from 0 to 80 min and centrifuged. The supernatant solution from each of these tubes was evaluated for the amount of Orange II present in them and the percent leak of Orange II was determined. As an example, the "10 min" sample tube was incubated for a period of 10 mins and immediately centrifuged and the supernatant was removed and analyzed for percent leak of Orange II. The "0 min" indicates that the sample was immediately centrifuged without incubation. The supernatant from these samples was evaluated for the number of moles of the dye present and this was compared with the number of moles of Orange II originally present in the aggregates. Figure 6 represents the percent leak of Orange II from the aggregates as a function of leaking (incubation) time. The percent leak of the dye increased from 18.2 to 24.4% as the incubation time was increased from 10 to 80 min. The figure also shows that there was no significant increase in the percent leak of the dye after a period of 50 min. Overall, the maximum percent leak of the dye from the aggregates was calculated to be 24.4%. Hence the retention efficiency of the aggregates was 75.6%, which is very similar to the unaggregated microgels. We also monitored if any additional dye leaked out upon addition of fresh buffer to the aggregates. To do this, we chose the sample of aggregates that leaked out the highest percent Orange II. As discussed above, that was the 80 min incubation sample. This sample leaked out 24.4% of the dye that was trapped in its structure. We added fresh 1.0 mL buffer to these aggregates, and redispersed them by vortexing. This solution was then incubated for an additional 80 min and centrifuged. The supernatant was analyzed by UV−vis and we observed no additional leaking. Additionally, we investigated the possibility of the microgels leaking Orange II at elevated temperature due to deswelling of aggregates above their LCST. We redispersed the 80 min incubation microgel aggregates in the supernatant solution containing the Orange II that leaked out of them. This solution was incubated for an additional 80 min on a hot plate at 50 °C and was immediately centrifuged for 30 min at 50°C by regulating the thermostat on the centrifuge. The supernatant was analyzed by UV−vis and the results from this prove that no more of the dye leaked out from the aggregates.

Langmuir Sorption Isotherm for the Removal of Orange II. Panels a and b in Figure 7 show the removal efficiency of the microgel aggregates as a function of the concentration of Orange II . Here, we pipetted 5, 10, 15, 20, 25, 30, and 35 μL of Orange II from the stock solution and added 300 μL of the 500 mg (10 mg/mL) BIS aggregates, the total aggregate mass was 1.56 mg. The total volume was maintained at 3 mL for all samples, using the pH 7 buffer solution and the solutions were incubated for five minutes. The solutions were centrifuged for 30 min and the supernatant was removed immediately and analyzed for the number of moles of the dye not "sorbed" on the aggregates (as explained in previous sections). A Langmuir isotherm model was used to fit the results from this experiment (eq 1), which gave a good fit with a R^2 of 0.9848. This yielded a maximum sorption of Orange II of 152.8 μmol/g (0.054 g Orange II/g aggregates) with a Langmuir coefficient of 0.01596 ± 0.00182 L/μmoles. Previously we

reported the maximum concentration of Orange II removed by the unaggregated microgels to be 139.9 μmol/g (0.049 g Orange II/g microgels).[1] We also plotted the sorption isotherm only considering the mass of the microgels in the aggregates, and fit the data with a Langmuir sorption model, which gave a good fit with an R^2 of 0.9853. In this case, the maximum Orange II sorbed was calculated to be 2871 μmol/g (1.006 g Orange II/g of microgels). The Langmuir coefficient was calculated as 0.016035 $\pm$ 0.0018 L/μmoles. Therefore, the microgels in the aggregates are capable of removing significantly more Orange II/g than the unaggregated microgels.

$$C_{i,s} = C_{i,smax} K_{ads} C_{i,mob} / (1 + K_{ads} C_{i,mob}) \quad (1)$$

where $C_{i,s}$ is the concentration of Orange II in aggregates (sorbent), $C_{i,mob}$ is the concentration of Orange II in mobile phase (in buffer after centrifugation), and K_{ads} is the Langmuir coefficient

Previously, pNIPAm based gels were used for adsorption of organic molecules like naphthalene disulfonic acid (NS-2).[68] The authors report the loading of NS-2 follows a Langmuir isotherm model, reporting R^2 values of ~0.97 and a maximum uptake of 13 mmol/L of gel (3.747 g NS-2/L of gel). Assuming the density of the gel to be 1.000 g/mL (assuming this will lead to the maximum loading capacity), the maximum loading capacity would be 0.0037 g NS-2/g of gel. In comparison, it is evident that the microgel aggregates reported in this submission achieved higher uptake efficiencies.

CONCLUSION

We report a system composed of pNIPAm microgel aggregates that are capable of removing a maximum of 73.1% of Orange II from water at elevated temperature. The results indicate that the Orange II uptake efficiency can be enhanced by increasing the BIS concentration from 100 to 500 mg, with minimal enhancement if the concentration of BIS is increased further. The results suggest that both the nature of the aggregate (hydrophobicity) and the aggregate number are important factors that affect uptake efficiency. Also, by exploiting the thermoresponsive nature of these aggregates and by increasing the number of heating/cooling cycles the removal efficiency can be enhanced. The overall retention efficiencies of the aggregates were determined by performing leaking studies, in which we determined that a maximum of 24.4% of the dye that was originally removed leaked out after an incubation time of 80 min, but there was minimal additional leaking after 50 min. The results for removal efficiencies as a function of the concentration of dye were fit with the Langmuir model and we evaluated the maximum sorption of Orange II on the aggregates to be 0.054 g Orange II/g aggregates with a Langmuir coefficient of 0.015960 $\pm$ 0.00182 L/μmol. When considering just the mass of the microgels, the Langmuir fit yielded a maximum value of Orange II sorbed of 2871 μmol/g (1.006 g Orange II/g of microgels). The Langmuir coefficient in this case was calculated as 0.016035 $\pm$ 0.0018 L/μmol. This study, combined with our previous report,[1] will serve as a basis for our future studies on using these systems to remediate water contaminated with naphthenic acids, polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and drug molecules/pharmaceuticals. These studies also serve as a useful tool to design efficient drug delivery systems.

ASSOCIATED CONTENT

Supporting Information. Calibration plot used for the calculation of removal efficiencies, uptake of Orange II as a function of aggregate concentration for the particle number experiment, UV−vis spectra for Orange II held at high temperature in the absence of aggregates, uptake studies of Orange II for multiple (three) heating and cooling cycles, a table detailing the results from aggregate counting experiment, light scattering data for thermoresponsivity of microgels. and DIC microscopy images of the aggregates. This material is available free of charge via the Internet at http://pubs.acs.org/.

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